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Immunological similarities between cytosolic and particulate tissue transglutaminase

C.R.L. Knight[1], R.C. Rees[2], B.M. Elliott[3] and M. Griffin[1]

[1]*Dept. of Life Sciences, Nottingham Polytechnic, Nottingham, NG11 8NS,* [2]*Dept. of Virology, University of Sheffield, Sheffield, S10 2RX* and [3]*ICI Central Toxicology Laboratories, Alderly Park, Macclesfield, SK10 4TJ, UK*

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At the present time it is uncertain whether or not the cytosolic and particulate forms of tissue transglutaminase are distinct and discrete enzymes. In this study a number of physical and immunological similarities between the two forms are demonstrated, indicating that they share some common epitopes, although their native confirmations may differ.

Cytosolic transglutaminase; Particulate transglutaminase

1. INTRODUCTION

Transglutaminases are a group of acyl-transferases that catalyse the post-translational cross-linking of proteins via the formation of $\epsilon(\gamma$-glutamyl)lysine bridges [1]. These enzymes exist in both extracellular forms (Factor XIII and prostate transglutaminase) and intracellular forms (epidermal, hair follicle and tissue transglutaminase) [2–4]. Tissue transglutaminase is thought to exist in two distinct forms, a predominantly soluble cytosolic enzyme and an insoluble particulate enzyme [5,6]. Although no role has as yet been confirmed for either form, it has been suggested that they may be involved in a number of calcium-mediated processes associated with the cell membrane and cytoskeleton [7–11], and with programmed cell death (apoptosis) [12]. Before the function of tissue transglutaminases can be fully understood, it is necessary to ascertain the relationship between the two forms since, at the present time, it is uncertain whether or not they are distinct and discrete enzymes. The aim of this study is to investigate any immunological similarities that may exist between the two enzyme forms.

2. EXPERIMENTAL

Cytosolic and particulate transglutaminase were semi-purified from rat liver homogenised to 20% (w/v) in buffered non-ionic detergent (1% Lubrol-PX in 0.25 M sucrose, 5 mM Tris, pH 7.4, 2 mM EDTA, 2 mM DTT, 1 mM PMSF) followed by anion exchange (Mono-Q column) chromatography [13]. Transglutaminase activity was measured by the Ca^{2+} dependent incorporation of [^{14}C]putrescine into *N,N*-dimethylcasein [13], and antigen was measured by a quantitative sandwich ELISA, utilising affinity purified goat anti-guinea pig liver cytosolic transglutaminase and rabbit anti-rat liver cytosolic transglutaminase [13]. After dialysis against 5 mM Tris, pH 7.4, 2 mM EDTA, 2 mM DTT, further purification of each enzyme form was carried out using a 3 ml affinity column of rabbit anti-cytosolic transglutaminase antibody linked to cyanogen bromide activated Sepharose. Antigen was eluted using 10% (v/v) dioxane in 0.25 M glycine/HCl, pH 2.5, then immediately neutralised with 2.5 M Tris, and desalted to remove the dioxane on a Pharmacia PD-10 column using 5 mM Tris, pH 7.4, 2 mM EDTA as the eluting buffer. Purified fractions were then freeze-dried. Non-denatured samples of affinity purified particulate and cytosolic transglutaminase were electrophoresed on 7.5% (w/v) acrylamide gels in non-reducing conditions and then Western blotted onto nitrocellulose [14]. Samples, denatured by boiling in 40% (w/v) SDS, 10% (v/v) 2-mercaptoethanol, were electrophoresed on 10% (w/v) acrylamide gels under reducing conditions [15] and then Western blotted onto nitrocellulose paper [14]. Western blots were immunoprobed with the affinity purified polyclonal anti-cytosolic transglutaminase antibody raised in goat, or mouse monoclonal anti-guinea pig cytosolic transglutaminase antibody, and visualised using a Biorad biotin/streptavidin HRP amplification system, as per the manufacturers directions. Prior to immunoprobing with the monoclonal antibody, some Western blots of non-reduced particulate enzyme were treated at 37°C for 1 h with 0.5 units in Tris-buffered saline (TBS) (100 mM Tris-HCl, pH 7.4, 150 mM NaCl) plus 10 mM $CaCl_2$, of either lipase, collagenase, mannosidase, glucosidase or galactosidase. As a control, Factor XIII (Boehringer), electrophoresed and Western blotted under comparable conditions, was also subjected to these treatments. Western blots of cytosolic and particulate transglutaminase obtained from non-denaturing polyacrylamide gels were also probed with the HRP labelled lectins Concanavalin A, *Triticum vulgaris* and *Bandeiraea simplicifolia*, in TBS plus 10 mM $CaCl_2$, 10 mM $MgCl_2$ and 10 mM $MnCl_2$.

3. RESULTS

As previously demonstrated [5,6], the cytosolic and particulate transglutaminases are easily separable by anion exchange (Mono-Q) chromatography, with the particulate form eluting at 0.15–0.25 M NaCl and the cytosolic form eluting at 0.35–0.45 M NaCl (Fig. 1A).

Correspondence address: M. Griffin, Dept. of Life Sciences, Nottingham Polytechnic, Nottingham NG11 8NS, UK

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Similarly, the two forms also differ in their elution pattern from a rabbit anti-cytosolic transglutaminase affinity column, with the cytosolic form eluting first, after 2 column volumes of eluent (6 ml), and the particulate form eluting after 4 column volumes (12 ml).

Antigen profiles of anion exchange eluent fractions indicated that both affinity-purified goat anti-cytosolic transglutaminase and rabbit anti-cytosolic transglutaminase cross-reacted with the particulate form (Fig. 1B). However, if the monoclonal anti-cytosolic transglutaminase antibody was used as either the capture antibody or the visualising antibody in the sandwich ELISA assay, only the cytosolic peak was observed (Fig. 1C), indicating non-reactivity of the monoclonal antibody with the particulate form.

Western blots of non-denaturing polyacrylamide gels, immunoprobed with polyclonal affinity purified goat anti-cytosolic transglutaminase confirmed the expected differences in the relative electrophoretic mobilities of the two forms but further demonstrated the cross-reactivity of the antibody with both the particulate and cytosolic forms. Similar blots immunoprobed with monoclonal anti-cytosolic transglutaminase only demonstrated cross-reactivity with the cytosolic form (Fig. 2). However, when Western blots obtained from denaturing SDS-polyacrylamide gels were immunoprobed with the monoclonal antibody, both cytosolic and particulate forms were demonstrated (Fig. 3). Furthermore, the relative mobilities and calculated molecular masses (87 kDa in each case) were found to be comparable.

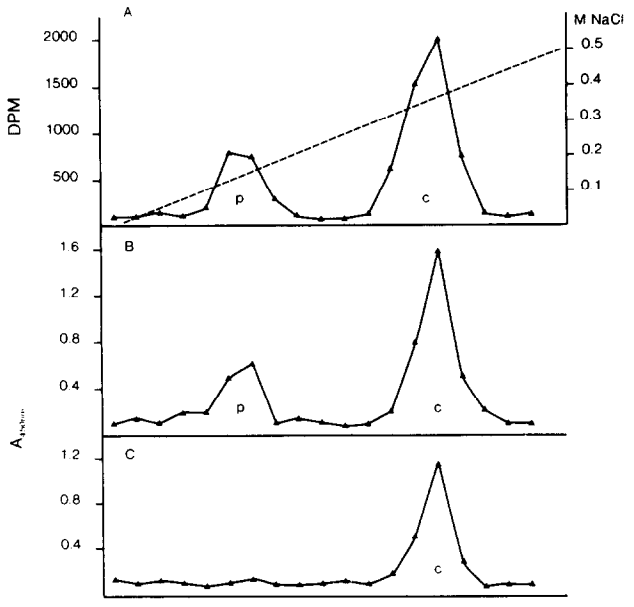

Fig. 1. Elution pattern of the particulate (p) and cytosolic (c) forms of transglutaminase on anion-exchange Mono-Q column (total elution volume 10 ml). (A) The enzyme activity profile. (B) Antigen profile using polyclonal antibody. (C) Antigen profile using monoclonal antibody.

Prior treatment of Western blots obtained from non-denaturing polyacrylamide gels with either lipase or collagenase also led to cross-reactivity of the particulate form with the monoclonal antibody (Fig. 2). The use of denaturing gel electrophoresis or the enzyme treatment of blots of the plasma transglutaminase Factor XIII did not produce a form capable of cross-reactivity with either the monoclonal or polyclonal antibodies (data not shown). Mannosidase, galactosidase or glucosidase treatment of Western blots did not produce a particulate form capable of cross-reacting with the monoclonal. Western blots of the particulate or cytosolic transglutaminase previously separated by non-denaturing polyacrylamide gel electrophoresis and probed with the HRP-labeled lectins, Concanavalin A,

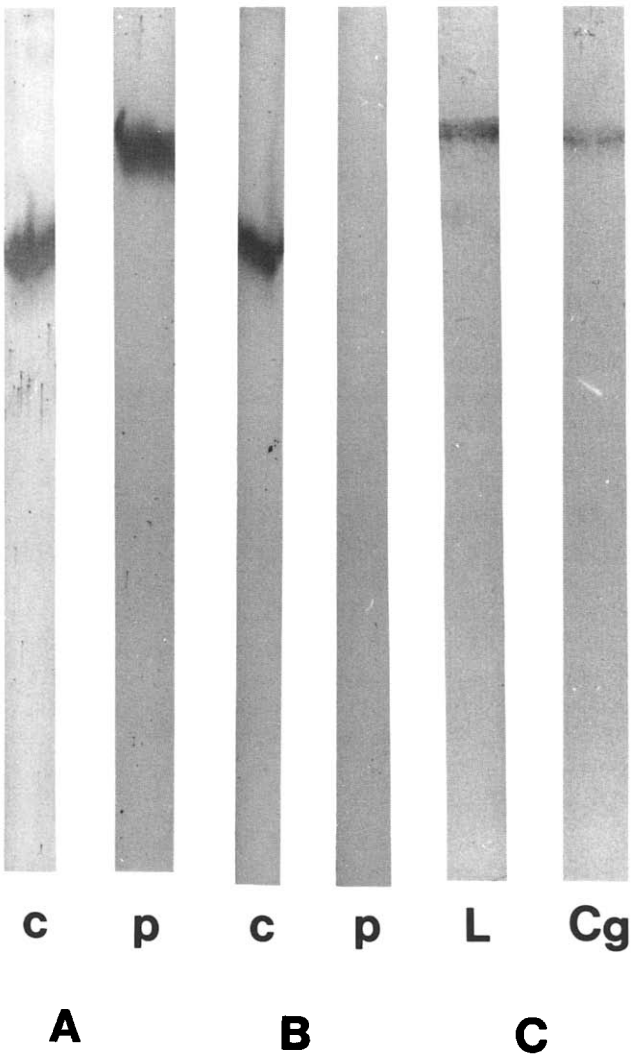

Fig. 2. Western blots of semi-purified (Mono-Q column) cytosolic (c) and particulate (p) transglutaminase following non-denaturing polyacrylamide gel electrophoresis. (A) Blots immunoprobed with polyclonal antibody. (B) Blots immunoprobed with monoclonal antibody. (C) Blots of the particulate immunoprobed with monoclonal antibody following treatment with lipase (L) and collagenase (Cg).

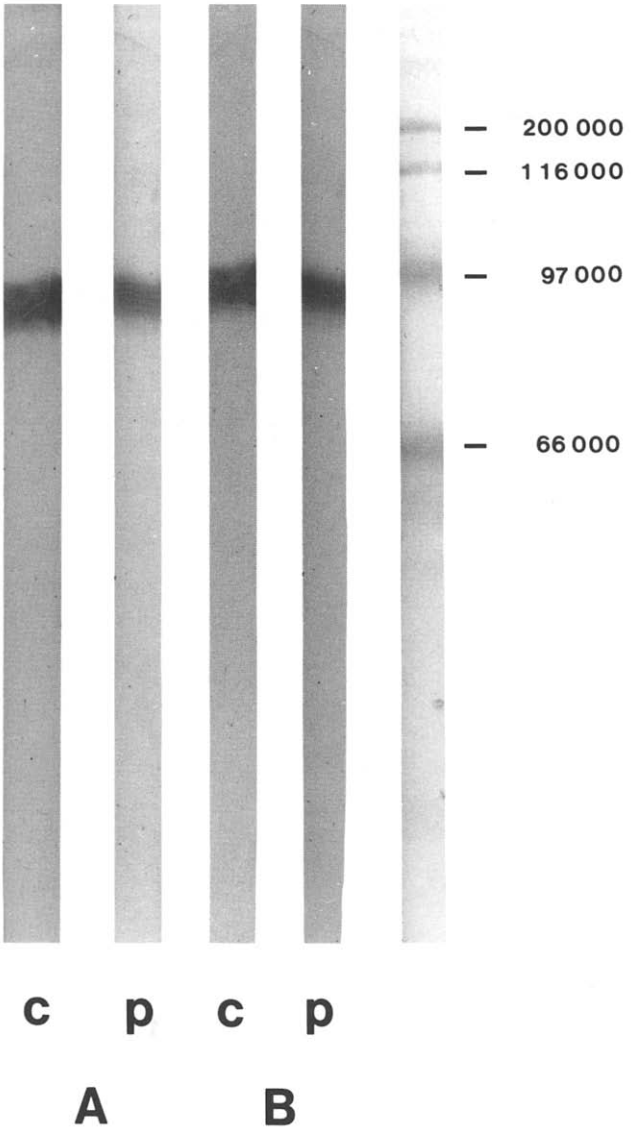

Fig. 3. Western blots of semi-purified (Mono-Q column) cytosolic (c) and particulate (p) transglutaminase following denaturing SDS-gel electrophoresis. (A) Blots immunoprobed with polyclonal antibody. (B) Blots immunoprobed with monoclonal antibody.

Triticum vulgaris and *Bandeiraea simplicifolia*, showed no reaction, confirming that mannose, glucose and galactose, respectively, were not components of either enzyme form.

4. DISCUSSION

Previous reports have indicated the molecular weight of the particulate transglutaminase, as calculated by size exclusion chromatography, to be 100 kDa, approximately 20 kDa greater than the known weight of the cytosolic form [5]. The data presented in this report indicate that when SDS-solubilised forms of the particulate and cytosolic transglutaminases are subjected to SDS-PAGE, their molecular masses appear to be comparable, at 87 kDa. On an immunological basis our data also indicate that there is clearly a number of similarities between the two enzymes. The sugars mannose, glucose and galactose do not appear to be present on either form, furthermore polyclonal antibodies raised against the cytosolic enzyme also reacted with the particulate form, and with a greater avidity, as suggested by their relative elution patterns from an anti-cytosolic transglutaminase affinity column. Studies with the monoclonal anti-cytosolic transglutaminase antibody indicated that this antibody does not cross-react with particulate transglutaminase when in its native form suggesting that the epitope is either missing from the protein or masked in some way. However, since SDS-denatured forms of the particulate enzyme were found to react with this monoclonal antibody, both the cytosolic and particulate enzymes must possess the epitope for this antibody. Since denaturation of the particulate enzyme reveals the epitope, the suggestion is that in the native enzyme the epitope is masked, either by further post-translational modification as found in epidermal transglutaminase [16], by the binding of extraneous material during purification or by differences in the tertiary structure of the two enzymes. Since SDS PAGE indicated that the two enzymes have comparable molecular masses, a result contrasting with that obtained from exclusion chromatography (85 kDa for the cytosolic and 100 kDa for the particulate) each of the latter proposals are possible. Previous workers have indicated that tissue transglutaminase has a high affinity for the extracellular matrix [17–19] and suggestions have been made that the binding of the cytosolic enzyme to collagen during cell fractionation may account for a number of its particulate properties. The binding of tissue transglutaminase to specific plasma membrane domains has also been noted [20]. The observation that treatment with either lipase or collagenase leads to cross-reactivity of non-denatured particulate transglutaminase with the monoclonal antibody suggests that the particulate enzyme does indeed associate with both lipids and collagen, and that this association may be responsible for masking the epitope.

In conclusion, our data obtained from immunological studies indicate that the two forms of tissue transglutaminase are far more closely related than previously documented, each carrying a number of similar epitopes.

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